20 Tricarboxylic Acid Cycle

The TCA cycle is frequently called the Krebs cycle because Sir Hans Krebs first formulated its reactions into a cycle. It is also called the "citric acid cycle" because citrate was one of the first compounds known to participate. The most common name for this pathway, the tricarboxylic acid or TCA cycle, denotes the involvement of the tricarboxylates citrate and isocitrate.

The major pathways of fuel oxidation generate acetyl CoA, which is the substrate for the TCA cycle. In the first step of the TCA cycle, the acetyl portion of acetyl CoA combines with the 4carbon intermediate oxaloacetate to form citrate (6 carbons), which is rearranged to form isocitrate. In the next two oxidative decarboxylation reactions, electrons are transferred to NAD⁺ to form NADH, and 2 molecules of electron-depleted CO2 are released. Subsequently, a high- energy phosphate bond in GTP is generated from substrate level phosphorylation. In the remaining portion of the TCA cycle, succinate is oxidized to oxaloacetate with the generation of one FAD(2H) and one NADH. The net reaction of the TCA cycle, which is the sum of the equations for individual steps, shows that the two carbons of the acetyl group have been oxidized to two molecules of CO₂, with conservation of energy as three molecules of NADH, one of FAD(2H), and one of GTP.

The **TCA cycle** (tricarboxylic acid cycle) accounts for over two thirds of the ATP generated from fuel oxidation. The pathways for oxidation of fatty acids, glucose, amino acids, acetate, and ketone bodies all generate **acetyl CoA**, which is the substrate for the TCA cycle. As the activated 2-carbon acetyl group is oxidized to two molecules of CO_2 , energy is conserved as NADH, FAD(2H), and GTP (Fig. 20.1). NADH and FAD(2H) subsequently donate electrons to O_2 via the electron transport chain, with the generation of ATP from oxidative phosphorylation. Thus, the TCA cycle is central to energy generation from cellular respiration.

Within the TCA cycle, the oxidative decarboxylation of α -ketoglutarate is catalyzed by the multisubunit α -ketoglutarate dehydrogenase complex, which contains the coenzymes thiamine-pyrophosphate, lipoate, and FAD. A similar complex, the pyruvate dehydrogenase complex (PDC), catalyzes the oxidation of pyruvate to acetyl CoA, thereby providing a link between the pathways of glycolysis and the TCA cycle (see Fig. 20.1)

The two-carbon acetyl group is the ultimate source of the electrons that are transferred to NAD⁺ and FAD and also the carbon in the two CO_2 molecules that are produced. Oxaloacetate is used and regenerated in each turn of the cycle (see Fig. 20.1). However, when cells use intermediates of the TCA cycle for



Fig. 20.1. Summary of the TCA cycle.

biosynthetic reactions, the carbons of oxaloacetate must be replaced by anaplerotic (filling up) reactions, such as the pyruvate carboxylase reaction.

The TCA cycle occurs in the mitochondrion, where its flux is tightly coordinated with the rate of the electron transport chain and oxidative phosphorylation through feedback regulation that reflects the demand for ATP. The rate of the TCA cycle is increased when ATP utilization in the cell is increased through the response of several enzymes to ADP levels, the NADH/ NAD + ratio, the rate of FAD(2H) oxidation or the Ca^{2+} concentration. For example, isocitrate dehydrogenase is allosterically activated by ADP.

There are two general consequences to impaired functioning of the TCA cycle: (1) an inability to generate ATP from fuel oxidation, and (2) an accumulation of TCA cycle precursors. For example, inhibition of pyruvate oxidation in the TCA cycle results in its reduction to lactate, which can cause a lactic acidosis. The most common situation leading to an impaired function of the TCA cycle is a relative lack of oxygen to accept electrons in the electron transport chain.



ТНЕ WAITING ROOM

Otto Shape, a 26-year-old medical student, has faithfully followed his diet and aerobic exercise program of daily tennis and jogging (see Chapter 19). He has lost a total of 33 lb and is just 23 lb from his college weight of 154 lb. His exercise capacity has markedly improved; he can run for a longer time at a faster pace before noting shortness of breath or palpitations of his heart. Even his test scores in his medical school classes have improved.

Ann O' Rexia suffers from anorexia nervosa (see Chapters 1, 3, and 9). In addition to a low body weight, decreased muscle mass, glycogen, and fat stores, she has iron-deficiency anemia (see Chapter 16). She has started to gain weight, and is trying a daily exercise program. However, she constantly feels weak and tired. When she walks, she feels pain in her calf muscles. On this visit to her nutritionist, they discuss the vitamin content of her diet, and its role in energy metabolism.



Al Martini has been hospitalized for congestive heart failure (see Chapter 8) and for head injuries sustained while driving under the influence of alcohol (Chapters 9 and 10). He completed an alcohol detoxification program, enrolled in a local Alcoholics Anonymous (AA) group, and began seeing a psychologist. During this time, his alcohol-related neurologic and cardiac manifestations of thiamine deficiency partially cleared. However, in spite of the support he was receiving, he began drinking excessive amounts of alcohol again while eating poorly. Three weeks later, he was readmitted with symptoms of "high output" heart failure.

I. **REACTIONS OF THE TCA CYCLE**

In the TCA cycle, the 2-carbon acetyl group of acetyl CoA is oxidized to 2 CO₂ molecules (see Fig. 20.1). The function of the cycle is to conserve the energy from this oxidation, which it accomplishes principally by transferring electrons from intermediates of the cycle to NAD^+ and FAD. The eight electrons donated by the acetyl group eventually end up in three molecules of NADH and one of FAD(2H) (Fig. 20.2). As a consequence, ATP can be generated from oxidative phosphorylation when NADH and FAD(2H) donate these electrons to O₂ via the electron transport chain.

Vitamins and minerals required for the TCA cycle and anaplerotic reactions Niacin (NAD⁺) Riboflavin (FAD) Pantothenate (CoA) Thiamine Biotin Mg^{2+} Ca²⁺ Fe²⁺ Phosphate

Fig. 20.2. The acetyl group of acetyl CoA. Acetyl CoA donates eight electrons to the TCA cycle, which are shown in blue, and two carbons. The high-energy bond is shown by a ~. The acetyl group is the ultimate source of the carbons in the two molecules of CO₂ that are produced, and the source of electrons in the one molecule of FAD(2H) and 3 molecules NADH, which have each accepted two electrons. However, the same carbon atoms and electrons that enter from one molecule of acetyl CoA do not leave as CO2, NADH, or FAD(2H) within the same turn of the cycle.

Synthases, such as citrate synthase, catalyze condensation of two organic molecules to form a carbon-carbon bond. Dehydrogenases, such as isocitrate dehydrogenase, are enzymes that remove electron-containing hydrogen or hydride atoms from a substrate and transfer them to electron-accepting coenzymes, such as NAD⁺ or FAD. Aconitase is an isomerase, an enzyme that catalyzes an internal rearrangement of atoms or electrons. In aconitase, a hydroxyl group is being transferred from one carbon to another. An iron cofactor in the enzyme facilitates this transfer.

Initially, the acetyl group is incorporated into citrate, an intermediate of the TCA cycle (Fig. 20.3). As citrate progresses through the cycle to oxaloacetate, it is oxidized by four dehydrogenases (isocitrate dehydrogenase, α -ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase), which transfer electrons to NAD⁺ or FAD. The isomerase aconitase rearranges electrons in citrate, thereby forming isocitrate, to facilitate an electron transfer to NAD⁺.

Although no O_2 is introduced into the TCA cycle, the two molecules of CO_2 produced have more oxygen than the acetyl group. These oxygen atoms are ultimately derived from the carbonyl group of acetyl CoA, two molecules of water added by fumarase and citrate synthase, and the PO_4^{2-} added to GDP.

The overall yield of energy-containing compounds from the TCA cycle is 3 NADH, 1 FAD(2H), and 1 GTP. The high-energy phosphate bond of GTP is generated from substrate level phosphorylation catalyzed by succinate thiokinase (succinyl CoA synthetase). As the NADH and FAD(2H) are reoxidized in the electron transport chain, approximately 2.5 ATP are generated for each NADH, and 1.5 ATP



Fig. 20.3. Reactions of the TCA cycle. The oxidation-reduction enzymes and coenzymes are shown in blue. Entry of the two carbons of acetyl CoA into the TCA cycle are indicated with blue dashed boxes. The carbons released as CO_2 are shown with black dashed boxes.

for the FAD(2H). Consequently, the net energy yield from the TCA cycle and oxidative phosphorylation is about 10 high-energy phosphate bonds for each acetyl group oxidized.

A. Formation and Oxidation of Isocitrate

The TCA cycle begins with condensation of the activated acetyl group and oxaloacetate to form the 6-carbon intermediate citrate, a reaction catalyzed by the enzyme citrate synthase (see Fig. 20.3). Because oxaloacetate is regenerated with each turn of the cycle, it is not really considered a substrate of the cycle, or a source of electrons or carbon.

In the next step of the TCA cycle, the hydroxyl (alcohol) group of citrate is moved to an adjacent carbon so that it can be oxidized to form a keto group. The isomerization of citrate to isocitrate is catalyzed by the enzyme aconitase, which is named for an intermediate of the reaction. The enzyme isocitrate dehydrogenase catalyzes the oxidation of the alcohol group and the subsequent cleavage of the carboxyl group to release CO₂ (an oxidative decarboxylation).

B. α-Ketoglutarate to Succinyl CoA

The next step of the TCA cycle is the oxidative decarboxylation of α -ketoglutarate to succinyl CoA, catalyzed by the α -ketoglutarate dehydrogenase complex (see Fig. 20.3). The dehydrogenase complex contains the coenzymes thiamine pyrophosphate, lipoic acid, and FAD.

In this reaction, one of the carboxyl groups of α -ketoglutarate is released as CO₂, and the adjacent keto group is oxidized to the level of an acid, which then combines with CoASH to form succinyl CoA (see Fig. 20.3). Energy from the reaction is conserved principally in the reduction state of NADH, with a smaller amount present in the high-energy thioester bond of succinyl CoA.

C. Generation of GTP

Energy from the succinyl CoA thioester bond is used to generate GTP from GDP and Pi in the reaction catalyzed by succinate thiokinase (see Fig. 20.3). This reaction is an example of substrate level phosphorylation. By definition, substrate level phosphorylation is the formation of a high-energy phosphate bond where none previously existed without the use of molecular O_2 (in other words, NOT oxidative phosphorylation). The high-energy phosphate bond of GTP is energetically equivalent to that of ATP, and can be used directly for energy-requiring reactions like protein synthesis.

D. Oxidation of Succinate to Oxaloacetate

Up to this stage of the TCA cycle, two carbons have been stripped of their available electrons and released as CO₂. Two pairs of these electrons have been transferred to 2 NAD⁺, and one GTP has been generated. However, two additional pairs of electrons arising from acetyl CoA still remain in the TCA cycle as part of succinate. The remaining steps of the TCA cycle transfer these two pairs of electrons to FAD and NAD⁺ and add H_2O , thereby regenerating oxaloacetate.

The sequence of reactions converting succinate to oxaloacetate begins with the oxidation of succinate to fumarate (see Fig. 20.3). Single electrons are transferred from the two adjacent -CH₂- methylene groups of succinate to an FAD bound to succinate dehydrogenase, thereby forming the double bond of fumarate. From the reduced enzyme-bound FAD, the electrons are passed into the electron transport chain. An OH⁻ group and a proton from water add to the double bond of fumarate, converting it to malate. In the last reaction of the TCA cycle, the alcohol group of malate is oxidized to a keto group through the donation of electrons to NAD⁺.

Otto Shape's exercise program increases his rate of ATP utilization and his rate of fuel oxidation in the TCA cycle. The TCA cycle generates NADH and FAD(2H), and the electron transport chain transfers electrons from NADH and FAD(2H) to O₂, thereby creating the electrochemical potential that drives ATP synthesis from ADP. As ATP is used in the cell, the rate of the electron transport chain increases. The TCA cycle and other fuel oxidative pathways respond by increasing their rates of NADH and FAD(2H) production.

Succinate thiokinase is also known as succinyl CoA synthetase. Both names refer to the reverse direction of the reaction, i.e., the conversion of succinate to the thioester succinyl CoA, utilizing energy from GTP. Synthases, such as citrate synthase, differ from synthetases in that synthetases cleave a high- energy phosphate bond in ATP, UTP, CTP, or GTP, and synthases do not.

From Figure 20.3, which enzymes in the TCA cycle release CO₂? How many moles of oxaloacetate are consumed in the TCA cycle for each mole of CO₂ produced?

The succinate to oxaloacetate sequence of reactions-oxidation through formation of a double bond, addition of water to the double bond, and oxidation of the resultant alcohol to a ketone is found in many oxidative pathways in the cell, such as the pathways for the oxidation of fatty acids, and oxidation of the branched chain amino acids.



Ann O'Rexia has been malnourished for some time, and has developed subclinical deficiencies of many vitamins, including riboflavin. The coenzymes FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) are synthesized from the vitamin riboflavin. Riboflavin is actively transported into cells, where the enzyme flavokinase adds a phosphate to form FMN. FAD synthetase then adds AMP to form FAD. FAD is the major coenzyme in tissues and is generally found tightly bound to proteins, with about 10% being covalently bound. Its turnover in the body is very slow, and people can live for long periods on low intakes without displaying any signs of a riboflavin deficiency.

Isocitrate dehydrogenase releases the first CO_2 , and α -ketoglutarate dehydrogenase releases the second CO_2 . There is no net consumption of oxaloacetate in the TCA cycle—the first step use an oxaloacetate, and the last step produces one. The utilization and regeneration of oxaloacetate is the "cycle" part of the TCA cycle.

One of **Otto Shape's** tennis partners told him that he had heard about a health food designed for athletes that contained succinate. The advertisement made the claim that succinate would provide an excellent source of energy during exercise because it could be metabolized directly without oxygen. Do you see anything wrong with this statement? With regeneration of oxaloacetate, the TCA cycle is complete; the chemical bond energy, carbon, and electrons donated by the acetyl group have been converted to CO_2 , NADH, FAD(2H), GTP, and heat.

II. COENZYMES OF THE TCA CYCLE

The enzymes of the TCA cycle rely heavily on coenzymes for their catalytic function. Isocitrate dehydrogenase and malate dehydrogenase use NAD⁺ as a coenzyme, and succinate dehydrogenase uses FAD. Citrate synthase catalyzes a reaction that uses a CoA derivative, acetyl CoA. The α -ketoglutarate dehydrogenase complex uses thiamine pyrophosphate, lipoate and FAD as bound coenzymes, and NAD⁺ and CoASH as substrates. Each of these coenzymes has unique structural features that enable it to fulfill its role in the TCA cycle.

A. FAD and NAD⁺

Both FAD and NAD⁺ are electron-accepting coenzymes. Why is FAD used in some reactions and NAD⁺ in others? Their unique structural features enable FAD and NAD⁺ to act as electron acceptors in different types of reactions, and play different physiological roles in the cell. FAD is able to accept single electrons (H•), and forms a half-reduced single electron intermediate (Fig. 20.4). It thus participates in reactions in which single electrons are transferred independently from two different atoms, which occurs in double bond formation (e.g., succinate to fumarate) and disulfide bond formation (e.g., lipoate to lipoate disulfide in the α -ketoglutarate



Fig. 20.4. One-electron steps in the reduction of FAD. When FAD and FMN accept single electrons, they are converted to the half-reduced semiquinone, a semistable free radical form. They can also accept two electrons to form the fully reduced form, $FADH_2$. However, in most dehydrogenases, $FADH_2$ is never formed. Instead, the first electron is shared with a group on the protein as the next electron is transferred. Therefore, in this text, overall acceptance of two electrons by FAD has been denoted by the more general abbreviation, FAD(2H).



Fig. 20.5. Oxidation and decarboxylation of isocitrate. The alcohol group (C—OH) is oxidized to a ketone, with the C—H electrons donated to NAD⁺ as the hydride ion. Subsequent electron shifts in the pyridine ring remove the positive charge. The H of the OH group dissociates into water as a proton, H^+ . NAD⁺, the electron acceptor, is reduced.

dehydrogenase reaction). In contrast, NAD⁺ accepts a pair of electrons as the hydride ion (H⁻), which is attracted to the carbon opposite the positively-charged pyridine ring (Fig. 20.5). This occurs, for example, in the oxidation of alcohols to ketones by malate dehydrogenase and isocitrate dehydrogenase. The nicotinamide ring accepts a hydride ion from the C-H bond, and the alcoholic hydrogen is released into the medium as a positively charged proton, H⁺.

The free radical, single-electron forms of FAD are very reactive, and FADH can lose its electron through exposure to water or the initiation of chain reactions. As a consequence, FAD must remain very tightly, sometimes covalently, attached to its enzyme while it accepts and transfers electrons to another group bound on the enzyme (Fig 20.6). Because FAD interacts with many functional groups on amino acid side chains in the active site, the $E^{0'}$ for enzyme-bound FAD varies greatly and can be greater or much less than that of NAD⁺. In contrast, NAD⁺ and NADH are more like substrate and product than coenzymes.

NADH plays a regulatory role in balancing energy metabolism that FAD(2H) cannot because FAD(2H) remains attached to its enzyme. Free NAD⁺ binds to a dehydrogenase and is reduced to NADH, which is then released into the medium where it can bind and inhibit a different dehydrogenase. Consequently, oxidative enzymes are controlled by the NADH/NAD⁺ ratio, and do not generate NADH faster than it can be reoxidized in the electron transport chain. The regulation of the TCA cycle and other pathways of fuel oxidation by the NADH/NAD⁺ ratio is part of the mechanism for coordinating the rate of fuel oxidation to the rate of ATP utilization.

B. Role of CoA in the TCA Cycle

CoASH, the acylation coenzyme, participates in reactions through the formation of a thioester bond between the sulfur (S) of CoASH and an acyl group (e.g., acetyl

FAD has been referred to as a married coenzyme, and NAD⁺ is its promiscuous cousin. FAD faithfully accepts only electrons from a substrate that is bound to the same enzyme (or enzyme complex), and donates these without leaving that enzyme. It does this repeatedly while still attached to its enzyme. NAD⁺, conversely, may accept electrons when bound to any dehydrogenase, and leaves the enzyme immediately afterward. It donates these electrons while bound to a different dehydrogenase, such as NADH dehydrogenase in the electron transport chain. It really gets around!

The claim that succinate oxidation could produce energy without oxygen is wrong. It was probably based on the fact that succinate is oxidized to fumarate by the donation of electrons to FAD. However, ATP can only be generated from this process when these electrons are donated to oxygen in the electron transport chain. The energy generated by the electron transport chain is used for ATP synthesis in the process of oxidative phosphorylation. After the covalently bound FAD(2H) is oxidized back to FAD by the electron transport chain, succinate dehydrogenase can oxidize another succinate molecule.



dehydrogenase

Fig. 20.6. Succinate dehydrogenase contains covalently bound FAD. As a consequence, succinate dehydrogenase and similar flavoproteins reside in the inner mitochondrial membrane where they can directly transfer electrons into the electron transport chain. The electrons are transferred from the covalently bound FAD to an Fe-S complex on the enzyme, and then to coenzyme Q in the electron transport chain (see Chapter 21). Thus, FAD does not have to dissociate from the enzyme to transfer its electrons. All the other enzymes of the TCA cycle are found in the mitochondrial matrix.



vitamin pantothenate in а sequence of reactions which phosphorylate pantothenate, add the sulfhydryl portion of CoA from cysteine, and then add AMP and an additional phosphate group from ATP (see Fig. 8.12). Pantothenate is widely distributed in foods (pantos means everywhere), so it is unlikely that Ann O'Rexia has developed a pantothenate deficiency. Although CoA is required in approximately 100 different reactions in mammalian cells, no Recommended Daily Allowance (RDA) has been established for pantothenate, in part because indicators have not yet been found which specifically and sensitively reflect a deficiency of this vitamin in the human. The reported symptoms of pantothenate deficiency (fatigue, nausea, and loss of appetite) are characteristic of vitamin deficiencies in general.

CoASH is synthesized from the



Fig. 20.8. Oxidative decarboxylation of α ketoglutarate. The α -ketoglutarate dehydrogenase complex oxidizes a-ketoglutarate to succinyl CoA. The carboxyl group is released as CO_2 . The keto group on the α -carbon is oxidized, and then forms the acyl CoA thioester, succinyl CoA. The α , β , γ , and δ on succinyl CoA refer to the sequence of atoms in α -ketoglutarate.



Fig. 20.7. Utilization of the high-energy thioester bond of acyl CoAs. Energy transformations are shown in blue. A. The energy released by hydrolysis of the thioester bond of acetyl CoA in the citrate synthase reaction contributes a large negative $\Delta G^{0'}$ to the forward direction of the TCA cycle. B. The energy of the succinyl CoA thioester bond is used for the synthesis of the high-energy phosphate bond of GTP.

CoA, succinyl CoA) (Fig. 20.7). The complete structure of CoASH and its vitamin precursor, pantothenate, is shown in Figure 8.12. A thioester bond differs from a typical oxygen ester bond because S, unlike O, does not share its electrons and participate in resonance formations. One of the consequences of this feature of sulfur chemistry is that the carbonyl carbon, the α -carbon and the β -carbon of the acyl group in a CoA thioester can be activated for participation in different types of reactions (e.g., in the citrate synthase reaction, the α -carbon methyl group is activated for condensation with oxaloacetate, see Figs. 20.3 and 20.7A). Another consequence is that the thioester bond is a high-energy bond that has a large negative $\Delta G^{0'}$ of hydrolysis (approximately-13 kcal/mole).

The energy from cleavage of the high-energy thioester bonds of succinyl CoA and acetyl CoA is used in two different ways in the TCA cycle. When the succinyl CoA thioester bond is cleaved by succinate thiokinase, the energy is used directly for activating an enzyme-bound phosphate that is transferred to GDP (see Fig. 20.7B). In contrast, when the thioester bond of acetyl CoA is cleaved in the citrate synthase reaction, the energy is released, giving the reaction a large negative $\Delta G^{0'}$ of -7.7 kcal/mole. The large negative $\Delta G^{0'}$ for citrate formation helps to keep the TCA cycle going in the forward direction.

C. The α -Ketoacid Dehydrogenase Complexes

The α -ketoglutarate dehydrogenase complex is one of a three-member family of similar α -keto acid dehydrogenase complexes. The other members of this family are the pyruvate dehydrogenase complex, and the branched chain amino acid α -keto acid dehydrogenase complex. Each of these complexes is specific for a different α keto acid structure. In the sequence of reactions catalyzed by the complexes, the α ketoacid is decarboxylated (i.e., releases the carboxyl group as CO₂) (Fig.20.8). The keto group is oxidized to the level of a carboxylic acid, and then combined with CoASH to form an acyl CoA thioester (e.g., succinyl CoA).

All of the α -ketoacid dehydrogenase complexes are huge enzyme complexes composed of multiple subunits of three different enzymes, E₁, E₂, and E₃ (Fig. 20.9). E_1 is an α -ketoacid decarboxylase which contains thiamine pyrophosphate (TPP); it cleaves off the carboxyl group of the α -keto acid. E₂ is a transacylase containing lipoate; it transfers the acyl portion of the α -keto acid from thiamine to CoASH. E₃ is dihydrolipoyl dehydrogenase, which contains



Fig. 20.9. Mechanism of α -keto acid dehydrogenase complexes (including α -ketoglutarate dehydrogenase, pyruvate dehydrogenase and the branched-chain α -keto acid dehydrogenase complex). **R** represents the portion of the α -ketoacid that begins with the β carbon. In α ketoglutarate, R is CH₂-CH₂-COOH. In pyruvate, R is CH₃. The individual steps in the oxidative decarboxylation of α -keto acids are catalyzed by three different subunits: E₁, α -ketoacid decarboxylase (α -ketoglutarate decarboxylase); E₂, transacylase (trans-succinylase), and E₃, dihydrolipoyl dehydrogenase. Circle 1: Thiamine pyrophosphate (TPP) on E_1 decarboxylates the α-ketoacid and forms a covalent intermediate with the remaining portion. Circle 2: The acyl portion of the α -keto acid is transferred by TPP on E₁ to lipoate on E₂, which is a transacylase. Circle 3: E_2 transfers the acyl group from lipoate to CoASH. This process has reduced the lipoyl disulfide bond to sulfhydryl groups (dihydrolipoyl). Circle 4: E_3 , dihydrolipoyl dehydrogenase (DH) transfers the electrons from reduced lipoate to its tightly bound FAD molecule, thereby oxidizing lipoate back to its original disulfide form. Circle 5: The electrons are then transferred from FAD(2H) to NAD⁺ to form NADH.

FAD; it transfers electrons from reduced lipoate to NAD⁺. The collection of 3 enzyme activities into one huge complex enables the product of one enzyme to be transferred to the next enzyme without loss of energy. Complex formation also increases the rate of catalysis because the substrates for E₂ and E₃ remain bound to the enzyme complex.

1. THIAMINE PYROPHOSPHATE IN THE α -KETOGLUTARATE DEHYDROGENASE COMPLEX

Thiamine pyrophosphate is synthesized from the vitamin thiamine by the addition of pyrophosphate (see Fig. 8.11). The pyrophosphate group binds magnesium, which binds to amino acid side chains on the enzyme. This binding is relatively weak for a coenzyme, so thiamine turns over rapidly in the body, and a deficiency can develop rapidly in individuals on a thiamine-free or low thiamine diet.

The general function of thiamine pyrophosphate is the cleavage of a carboncarbon bond next to a keto group. In the α -ketoglutarate, pyruvate, and branched chain α -keto acid dehydrogenase complexes, the functional carbon on the thiazole ring forms a covalent bond with the α -keto carbon, thereby cleaving the bond between the α -keto carbon and the adjacent carboxylic acid group (see Fig. 8.11 for the mechanism of this reaction). Thiamine pyrophosphate is also a coenzyme for transketolase in the pentose phosphate pathway, where it similarly cleaves the carbon-carbon bond next to a keto group. In thiamine deficiency, α -ketoglutarate, pyruvate, and other α -keto acids accumulate in the blood.

2. LIPOATE

Lipoate is a coenzyme found only in α -keto acid dehydrogenase complexes. It is synthesized in the human from carbohydrate and amino acids, and does not require

The E⁰ for FAD accepting electrons is -0.20 (see Table 19.4). The E⁰ for NAD⁺ accepting electrons is -0.32. Thus, transfer of electrons from FAD(2H) to NAD⁺ is energetically unfavorable. How do the *a*-keto acid dehydrogenase complexes allow this electron transfer to occur?

In Al Martini's heart failure, which is caused by a dietary deficiency of the vitamin thiamine, pyruvate dehydrogenase, a-ketoglutarate dehydrogenase, and the branched chain α -keto acid dehydrogenase complexes are less functional than normal. Because heart muscle, skeletal muscle, and nervous tissue have a high rate of ATP production from the NADH produced by the oxidation of pyruvate to acetyl CoA and of acetyl CoA to CO₂ in the TCA cycle, these tissues present with the most obvious signs of thiamine deficiency.

In Western societies, gross thiamine deficiency is most often associated with alcoholism. The mechanism for active absorption of thiamine is strongly and directly inhibited by alcohol. Subclinical deficiency of thiamine from malnutrition or anorexia may be common in the general population and is usually associated with multiple vitamin deficiencies.

The E^{0'} values were calculated in a test tube under standard conditions. When FAD is bound to an enzyme, as it is in the α -keto acid dehydrogenase complexes, amino acid side chains can alter its E^{0'} value. Thus, the transfer of electrons from the bound FAD(2H) to NAD⁺ in dihydrolipoyl dehydrogenase is actually energetically favorable.

Arsenic poisoning is caused by the presence of a large number of different arsenious compounds that are effective metabolic inhibitors. Acute accidental or intentional arsenic poisoning requires high doses and involves arsenate (AsO_4^{2-}) and arsenite (AsO^{2-}) . Arsenite, which is 10 times more toxic than arsenate, binds to neighboring sulfhydryl groups, such as those in dihydrolipoate and in nearby cysteine pairs (vicinal) found in α keto acid dehydrogenase complexes and in succinic dehydrogenase. Arsenate weakly inhibits enzymatic reactions involving phosphate, including the enzyme glyceraldehyde 3-P dehydrogenase in glycolysis (see Chapter 22). Thus both aerobic and anaerobic ATP production can be inhibited. The low doses of arsenic compounds found in water supplies are a major public health concern, but are associated with increased risk of cancer rather than direct toxicity.



Fig. 20.10. Function of lipoate. Lipoate is attached to the ϵ -amino group on the lysine side chain of the tranacylase enzyme (E₂). The oxidized lipoate disulfide form is reduced as it accepts the acyl group from thiamine pyrophosphate (TPP) attached to E₁. The example shown is for the α -ketoglutarate dehydrogenase complex.

a vitamin precursor. Lipoate is attached to the transacylase enzyme through its carboxyl group, which is covalently bound to the terminal -NH₂ of a lysine in the protein (Fig. 20.10). At its functional end, lipoate contains a disulfide group that accepts electrons when it binds the acyl fragment of α -ketoglutarate. It can thus act like a long flexible -CH₂- arm of the enzyme that reaches over to the decarboxylase to pick up the acyl fragment from thiamine and transfer it to the active site containing bound CoASH. It then swings over to dihydrolipoyl dehydrogenase to transfer electrons from the lipoyl sulfhydryl groups to FAD.

3. FAD AND DIHYDROLIPOYL DEHYDROGENASE

FAD on dihydrolipoyl dehydrogenase accepts electrons from the lipoyl sulfhydryl groups and transfers them to bound NAD⁺. FAD thus accepts and transfers electrons without leaving its binding site on the enzyme. The direction of the reaction is favored by interactions of FAD with groups on the enzyme, which change its reduction potential and by the overall release of energy from cleavage and oxidation of α -ketoglutarate.

III. ENERGETICS OF THE TCA CYCLE

Like all metabolic pathways, the TCA cycle operates with an overall net negative $\Delta G^{0'}$ (Fig 20.11). The conversion of substrates to products is, therefore, energetically favorable. However, some of the reactions, such as the malate dehydrogenase reaction, have a positive value.



Fig. 20.11. Approximate $\Delta G^{0'}$ values for the reactions in the TCA cycle, given for the forward direction. The reactions with large negative $\Delta G^{0'}$ values are shown in blue. The standard free energy ($\Delta G^{0'}$) refers to the free energy change for conversion of 1 mole of substrate to 1 mole of product under standard conditions (see Chapter 19).

A. Overall Efficiency of the TCA Cycle

The reactions of the TCA cycle are extremely efficient in converting energy in the chemical bonds of the acetyl group to other forms. The total amount of energy available from the acetyl group is about 228 kcal/mole (the amount of energy that could be released from complete combustion of 1 mole of acetyl groups to CO_2 in a bomb calorimeter). The products of the TCA cycle (NADH, FAD(2H), and GTP) contain about 207 kcal (Table 20.1). Thus, the TCA cycle reactions are able to conserve about 90% of the energy available from the oxidation of acetyl CoA.

B. Thermodynamically and Kinetically Reversible and Irreversible reactions

Three reactions in the TCA cycle have large negative values for $\Delta G^{0'}$ that strongly favor the forward direction: the reactions catalyzed by citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase (see Fig. 20.11). Within the TCA cycle, these reactions are physiologically irreversible for two reasons: the products do not rise to high enough concentrations under physiological conditions to overcome the large negative $\Delta G^{0'}$ values, and the enzymes involved catalyze the reverse reaction very slowly. These reactions make the major contribution to the overall negative $\Delta G^{0'}$ for the TCA cycle, and keep it going in the forward direction.

In contrast to these irreversible reactions, the reactions catalyzed by aconitase and malate dehydrogenase have a positive $\Delta G^{0'}$ for the forward direction, and are thermodynamically and kinetically reversible. Because aconitase is rapid in both directions, equilibrium values for the concentration ratio of products to substrates is maintained, and the concentration of citrate is about 20 times that of isocitrate. The accumulation of citrate instead of isocitrate facilitates transport of excess citrate to the cytosol, where it can provide a source of acetyl CoA for pathways like fatty acid and cholesterol synthesis. It also allows citrate to serve as an inhibitor of citrate synthase when flux through isocitrate dehydrogenase is decreased. Likewise, the equilibrium constant of the malate dehydrogenase reaction favors the accumulation of malate over oxaloacetate, resulting in a low oxaloacetate concentration that is influenced by the NADH/NAD⁺ ratio. Thus, there is a net flux of oxaloacetate towards malate in the liver during fasting (due to fatty acid oxidation, which raises the NADH/NAD⁺ ratio), and malate can then be transported out of the mitochondria to provide a substrate for gluconeogenesis.

IV. REGULATION OF THE TCA CYCLE

The oxidation of acetyl CoA in the TCA cycle and the conservation of this energy as NADH and FAD(2H) is essential for generation of ATP in almost all tissues in the body. In spite of changes in the supply of fuels, type of fuels in the blood, or rate of ATP utilization, cells maintain ATP homeostasis (a constant level of ATP). The rate of the TCA cycle, like that of all fuel oxidation pathways, is principally regulated to correspond to the rate of the electron transport chain, which is regulated by the ATP/ADP ratio and the rate of ATP utilization (see Chapter 21). The major sites of regulation are shown in Fig 20.12.

Two major messengers feed information on the rate of ATP utilization back to the TCA cycle: (a) the phosphorylation state of ATP, as reflected in ATP and ADP levels, and (b) the reduction state of NAD⁺, as reflected in the ratio of NADH/NAD⁺. Within the cell, even within the mitochondrion, the total adenine nucleotide pool (AMP, ADP, plus ATP) and the total NAD pool (NAD⁺ plus NADH) are relatively constant. Thus, an increased rate of ATP utilization results in a small decrease of ATP concentration and an increase of ADP. Likewise, increased NADH oxidation to NAD⁺ by the electron transport chain increases the rate of pathways producing NADH. Under normal physiological conditions, the TCA cycle and other

Table 20.1. Energy Yield of the TCACycle

kcal/mole	
3 NADH: 3 × 53 1 FAD(2H) 1 GTP Sum	= 159 = 41 = 7 = 207

Chapter 19 explains the values given for energy yield from NADH and FAD(2H).

The net standard free energy change for the TCA cycle, $\Delta G^{0'}$, can be calculated from the sum of the $\Delta G^{0'}$, values for the individual reactions. The $\Delta G^{0'}$, -13 kcal, is the amount of energy lost as heat. It can be considered the amount of energy spent to ensure that oxidation of the acetyl group to CO₂ goes to completion. This value is surprisingly small. However, oxidation of NADH and FAD(2H) in the electron transport chain helps to make acetyl oxidation more energetically favorable and pull the TCA cycle forward.

Otto Shape had difficulty losing weight because human fuel utilization is too efficient. His adipose tissue fatty acids are being converted to acetyl CoA, which is being oxidized in the TCA cycle, thereby generating NADH and FAD(2H). The energy in these compounds is used for ATP synthesis from oxidative phosphorylation. If his fuel utilization were less efficient and his ATP yield were lower, he would have to oxidize much greater amounts of fat to get the ATP he needs for exercise.

As Otto Shape exercises, his myosin ATPase hydrolyzes ATP to provide the energy for movement of myofibrils. The decrease of ATP and increase of ADP stimulates the electron transport chain to oxidize more NADH and FAD(2H). The TCA cycle is stimulated to provide more NADH and FAD(2H) to the electron transport chain. The activation of the TCA cycle occurs through a decrease of the NADH/NAD⁺ ratio, an increase of ADP concentration, and an increase of $Ca^{2+}.$ Although regulation of the transcription of genes for TCA cycle enzymes is too slow to respond to changes of ATP demands during exercise, the number and size of mitochondria increase during training. Thus, Otto Shape is increasing his capacity for fuel oxidation as he trains.



Fig. 20.12. Major regulatory interactions in the TCA cycle. The rate of ATP hydrolysis controls the rate of ATP synthesis, which controls the rate of NADH oxidation in the electron transport chain (ETC). All NADH and FAD(2H) produced by the cycle donate electrons to this chain (shown on the right). Thus, oxidation of acetyl CoA in the TCA cycle can go only as fast as electrons from NADH enter the electron transport chain, which is controlled by the ATP and ADP content of the cells. The ADP and NADH concentrations feed information on the rate of oxidative phosphorylation back to the TCA cycle. Isocitrate dehydrogenase (DH), α -ketoglutarate dehydrogenase (DH), and malate dehydrogenase (DH) are inhibited by increased NADH concentration. The NADH/NAD⁺ ratio changes the concentration of oxaloacetate. Citrate is a product inhibitor of citrate synthase. ADP is an allosteric activator of isocitrate dehydrogenase. During muscular contraction, increased Ca²⁺ concentrations activate isocitrate DH and α -ketoglutarate dehydrogenase (as well as pyruvate dehydrogenase).

oxidative pathways respond so rapidly to increased ATP demand that the ATP concentration does not significantly change.

A. Regulation of Citrate Synthase

The principles of pathway regulation are summarized in Table 20.2. In pathways subject to feedback regulation, the first step of the pathway must be regulated so that

Table 20.2. Generalizations on the Regulation of Metabolic Pathways

- 1. Regulation matches function. The type of regulation use depends on the function of the pathway. Tissue-specific isozymes may allow the features of regulatory enzymes to match somewhat different functions of the pathway in different tissues.
- Regulation of metabolic pathways occurs at rate-limiting steps, the slowest steps, in the pathway. These are reactions in which a small change of rate will affect the flux through the whole pathway.
- 3. Regulation usually occurs at the first committed step of a pathway or at metabolic branchpoints. In human cells, most pathways are interconnected with other pathways and have regulatory enzymes for every branchpoint.
- 4. Regulatory enzymes often catalyze physiologically irreversible reactions. These are also the steps that differ in biosynthetic and degradative pathways.
- 5. Many pathways have "feedback" regulation, that is, the endproduct of the pathway controls the rate of its own synthesis. Feedback regulation may involve inhibition of an early step in the pathway (feedback inhibition) or regulation of gene transcription.
- 6. Human cells use compartmentation to control access of substrate and activators or inhibitors to different enzymes.
- 7. Hormonal regulation integrates responses in pathways requiring more than one tissue. Hormones generally regulate fuel metabolism by: a. Changing the phosphorylation state of enzymes.
 - b. Changing the amount of enzyme present by changing its rate of synthesis (often induction or repression of mRNA synthesis) or degradation.
 - c. Changing the concentration of an activator or inhibitor.

precursors flow into alternate pathways if product is not needed. Citrate synthase, which is the first enzyme of the TCA cycle, is a simple enzyme that has no allosteric regulators. Its rate is controlled principally by the concentration of oxaloacetate, its substrate, and the concentration of citrate, a product inhibitor, competitive with oxaloacetate.(see Fig. 20.12). The malate-oxaloacetate equilibrium favors malate, so the oxaloacetate concentration is very low inside the mitochondrion, and is below the $K_{m,app}$ (see Chapter 9, section I.A.4) of citrate synthase. When the NADH/NAD⁺ ratio decreases, the ratio of oxaloacetate to malate increases. When isocitrate dehydrogenase is activated, the concentration of citrate decreases, thus relieving the product inhibition of citrate synthase. Thus, both increased oxaloacetate and decreased citrate levels regulate the response of citrate synthase to conditions established by the electron transport chain and oxidative phosphorylation. In the liver, the NADH/NAD⁺ ratio helps determine whether acetyl CoA enters the TCA cycle or goes into the alternate pathway for ketone body synthesis.

B. Allosteric Regulation of Isocitrate Dehydrogenase

Another generalization that can be made about regulation of metabolic pathways is that it occurs at the enzyme that catalyzes the rate-limiting (slowest) step in a pathway (see Table 20.2). Isocitrate dehydrogenase is considered one of the ratelimiting steps of the TCA cycle, and is allosterically activated by ADP and inhibited by NADH (Fig. 20.13). In the absence of ADP, the enzyme exhibits positive cooperativity; as isocitrate binds to one subunit, other subunits are converted to an active conformation (see Chapter 9, section III.A on allosteric enzymes). In the presence of ADP, all of the subunits are in their active conformation, and isocitrate binds more readily. Consequently, the $K_{m,app}$ (the S_{0.5}) shifts to a much lower value. Thus, at the concentration of isocitrate found in the mitochondrial matrix, a small change in the concentration of ADP can produce a large change in the rate of the isocitrate dehydrogenase reaction. Small changes in the concentration of the product, NADH, and of the cosubstrate, NAD⁺, also affect the rate of the enzyme more than they would a nonallosteric enzyme.

C. Regulation of α-Ketoglutarate Dehydrogenase

The α -ketoglutarate dehydrogenase complex, although not an allosteric enzyme, is product-inhibited by NADH and succinyl CoA, and may also be inhibited by GTP (see Fig. 20.12). Thus, both α -ketoglutarate dehydrogenase and isocitrate dehydrogenase respond directly to changes in the relative levels of ADP and hence the rate at which NADH is oxidized by electron transport. Both of these enzymes are also activated by Ca²⁺. In contracting heart muscle, and possibly other muscle tissues, the release of Ca²⁺ from the sarcoplasmic reticulum during muscle contraction may provide an additional activation of these enzymes when ATP is being rapidly hydrolyzed.

D. Regulation of TCA Cycle Intermediates

Regulation of the TCA cycle serves two functions: it ensures that NADH is generated fast enough to maintain ATP homeostasis and it regulates the concentration of TCA cycle intermediates. For example, in the liver, a decreased rate of isocitrate dehydrogenase increases citrate concentration, which stimulates citrate efflux to the cytosol. A number of regulatory interactions occur in the TCA cycle, in addition to those mentioned above, that control the levels of TCA intermediates and their flux into pathways that adjoin the TCA cycle.

V. PRECURSORS OF ACETYL CoA

Compounds enter the TCA cycle as acetyl CoA or as an intermediate that can be converted to malate or oxaloacetate. Compounds that enter as acetyl CoA are



Fig. 20.13. Allosteric regulation of isocitrate dehydrogenase (ICDH). Isocitrate dehydrogenase has eight subunits, and two active sites. Isocitrate, NAD⁺, and NADH bind in the active site; ADP and Ca2+ are activators and bind to separate allosteric sites. A. A graph of velocity versus isocitrate concentration shows positive cooperativity (sigmoid curve) in the absence of ADP. The allosteric activator ADP changes the curve into one closer to a rectangular hyperbola, and decreases the K_m (S_{0.5}) for isocitrate. B. The allosteric activation by ADP is not an all-or-nothing response. The extent of activation by ADP depends on its concentration. C. Increases in the concentration of product, NADH, decrease the velocity of the enzyme through effects on the allosteric activation.

Acetate (acetic acid) is present in the diet, and can be produced from the oxidation of ethanol. Roman soldiers carried vinegar, a dilute solution of acetic acid. The acidity of the vinegar made it a relatively safe source of drinking water because many kinds of pathogenic bacteria do not grow well in acid solutions. The acetate, which is activated to acetyl CoA, provided an excellent fuel for muscular exercise.

> CH₃ -**Pyruvate** NAD⁺ Thiamine – (P) P CoASH Lipoate FAD CO Pyruvate dehydrogenase NADH complex + HCH₂ ~ SCoA Acetyl CoA

Fig. 20.15. Pyruvate dehydrogenase complex

(PDC) catalyzes oxidation of the α -ketoacid

pyruvate to acetyl CoA.

oxidized to CO_2 . Compounds that enter as TCA cycle intermediates replenish intermediates that have been used in biosynthetic pathways, such as gluconeogenesis or heme synthesis, but cannot be fully oxidized to CO_2 .

A. Sources of Acetyl CoA

Acetyl CoA serves as a common point of convergence for the major pathways of fuel oxidation. It is generated directly from the β -oxidation of fatty acids and degradation of the ketone bodies β -hydroxybutyrate and acetoacetate (Fig. 20.14). It is also formed from acetate, which can arise from the diet or from ethanol oxidation. Glucose and other carbohydrates enter glycolysis, a pathway common to all cells, and are oxidized to pyruvate. The amino acids alanine and serine are also converted to pyruvate. Pyruvate is oxidized to acetyl CoA by the pyruvate dehydrogenase complex. A number of amino acids, such as leucine and isoleucine are also oxidized to acetyl CoA. Thus, the final oxidation of acetyl CoA to CO₂ in the TCA cycle is the last step in all the major pathways of fuel oxidation.

B. Pyruvate Dehydrogenase Complex

The pyruvate dehydrogenase complex (PDC) oxidizes pyruvate to acetyl CoA, thus linking glycolysis and the TCA cycle. In the brain, which is dependent on the oxidation of glucose to CO_2 to fulfill its ATP needs, regulation of the PDC is a life and death matter.

1. STRUCTURE OF PDC

PDC belongs to the α -ketoacid dehydrogenase complex family and, thus, shares structural and catalytic features with the α -ketoglutarate dehydrogenase complex and the branched chain α -ketoacid dehydrogenase complex (Fig. 20.15). It contains the same three basic types of catalytic subunits: (1) pyruvate decarboxylase subunits that bind thiamine-pyrophosphate (E₁); (2) transacetylase subunits that bind lipoate (E₂), and (3) dihyrolipoyl dehydrogenase subunits that bind FAD (E₃) (see Fig. 20.9). Although the E₁ and E₂ enzymes in PDC are relatively specific for pyruvate, the same dihydrolipoyl dehydrogenase participates in all of the α -ketoacid dehydrogenase



Fig. 20.14. Origin of the acetyl group from various fuels. Acetyl CoA is derived from the oxidation of fuels. The portions of fatty acids, ketone bodies, glucose, pyruvate, the amino acid alanine, and ethanol that are converted to the acetyl group of acetyl CoA are shown in blue.

complexes. In addition to these three types of subunits, the PDC complex contains one additional catalytic subunit, protein X, which is a transacetylase. Each functional component of the PDC complex is present in multiple copies (e.g., bovine heart PDC has 30 subunits of E_1 , 60 subunits of E_2 , and 6 subunits each of E_3 and X). The E_1 enzyme is itself a tetramer of two different types of subunits, α and β .

2. REGULATION OF PDC

PDC activity is controlled principally through phosphorylation by pyruvate dehydrogenase kinase, which inhibits the enzyme, and dephosphorylation by pyruvate dehydrogenase phosphatase, which activates it (Fig. 20.16). Pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase are regulatory subunits within the PDC complex and act only on the complex. PDC kinase transfers a phosphate from ATP to specific serine hydroxyl (ser-OH) groups on pyruvate decarboxylase (E₁). PDC phosphatase removes these phosphate groups by hydrolysis. Phosphorylation of just one serine on the PDC E₁ α subunit can decrease its activity by over 99%. PDC kinase is present in complexes as tissue-specific isozymes that vary in their regulatory properties.

PDC kinase is, itself, inhibited by ADP and pyruvate. Thus, when rapid ATP utilization results in an increase of ADP, or when activation of glycolysis increases pyruvate levels, PDC kinase is inhibited, and PDC remains in an active, nonphosphorylated form. PDC phosphatase requires Ca^{2+} for full activity. In the heart, increased intramitochondrial Ca^{2+} during rapid contraction activates the phosphatase, thereby increasing the amount of active, nonphosphorylated PDC.

PDC is also regulated through inhibition by its products, acetyl CoA and NADH. This inhibition is stronger than regular product inhibition because their binding to

Deficiencies of the pyruvate dehydrogenase complex (PDC) are among the most common inherited diseases leading to lacticacidemia and, like pyruvate carboxylase deficiency, are grouped into the category of Leigh's disease. In its severe form, PDC deficiency presents with overwhelming lactic acidosis at birth, with death in the neonatal period. In a second form of presentation, the lactic academia is moderate, but there is profound psychomotor retardation with increasing age. In many cases, concomitant damage to the brain stem and basal ganglia lead to death in infancy. The neurological symptoms arise because the brain has a very limited ability to use fatty acids as a fuel, and is, therefore, dependent on glucose metabolism for its energy supply.

The most common PDC genetic defects are in the gene for the α subunit of E₁. The E₁ α -gene is X-linked. Because of its importance in central nervous system metabolism, pyruvate dehydrogenase deficiency is a problem in both males and females, even if the female is a carrier. For this reason, it is classified as an X-linked dominant disorder.



Fig. 20.16. Regulation of pyruvate dehydrogenase complex (PDC). PDC kinase, a subunit of the enzyme, phosphorylates PDC at a specific serine residue, thereby converting PDC to an inactive form. The kinase is inhibited by ADP and pyruvate. PDC phosphatase, another subunit of the enzyme, removes the phosphate, thereby activating PDC. The phosphatase is activated by Ca^{2+} . When the substrates, pyruvate and CoASH, are bound to PDC, the kinase activity is inhibited and PDC is active. When the products acetyl CoA and NADH bind to PDC, the kinase activity is stimulated, and the enzyme is phosphorylated to the inactive form. E_1 and the kinase exist as tissue-specific isozymes with overlapping tissue specificity, and somewhat different regulatory properties.

PDC stimulates its phosphorylation to the inactive form. The substrates of the enzyme, CoASH and NAD⁺, antagonize this product inhibition. Thus, when an ample supply of acetyl CoA for the TCA cycle is already available from fatty acid oxidation, acetyl CoA and NADH build up and dramatically decrease their own further synthesis by PDC.

PDC can also be rapidly activated through a mechanism involving insulin, which plays a prominent role in adipocytes. In many tissues, insulin may, slowly over time, increase the amount of pyruvate dehydrogenase complex present.

The rate of other fuel oxidation pathways that feed into the TCA cycle is also increased when ATP utilization increases. Insulin, other hormones and diet control the availability of fuels for these oxidative pathways.

VI. TCA CYCLE INTERMEDIATES AND ANAPLEROTIC REACTIONS

A. TCA Cycle Intermediates are Precursors for Biosynthetic Pathways

The intermediates of the TCA cycle serve as precursors for a variety of different pathways present in different cell types (Fig. 20.17). This is particularly important in the central metabolic role of the liver. The TCA cycle in the liver is often called an "open cycle" because there is such a high efflux of intermediates. After a high carbohydrate meal, citrate efflux and cleavage to acetyl CoA provides acetyl units for cytosolic fatty acid synthesis. During fasting, gluconeogenic precursors are converted to malate, which leaves the mitochondria for cytosolic gluconeogenesis. The liver also uses TCA cycle intermediates to synthesize carbon skeletons of amino acids. Succinyl CoA may be removed from the TCA cycle to form heme in cells of the liver and bone marrow. In the brain, α -ketoglutarate is converted to glutamate and then to γ -aminobutyric acid (GABA), a neurotransmitter. In skeletal muscle, α -ketoglutarate is converted to glutamine, which is transported through the blood to other tissues.

B. Anaplerotic Reactions

Removal of any of the intermediates from the TCA cycle removes the 4 carbons that are used to regenerate oxaloacetate during each turn of the cycle. With depletion of oxaloacetate, it is impossible to continue oxidizing acetyl CoA. To enable the TCA



Fig. 20.17. Efflux of intermediates from the TCA cycle. In the liver, TCA cycle intermediates are continuously withdrawn into the pathways of fatty acid synthesis, amino acid synthesis, gluconeogenesis, and heme synthesis. In brain, α -ketoglutarate is converted to glutamate and GABA, both neurotransmitters.

Pyruvate, citrate, α-ketoglutarate and malate, ADP, ATP, and phosphate (as well as many other compounds) have specific transporters in the inner mitochondrial membrane that transport compounds between the mitochondrial matrix and cytosol in exchange for a compound of similar charge. In contrast, CoASH, acetyl CoA, other CoA derivatives, NAD⁺ and NADH, and oxaloacetate, are not transported at a metabolically significant rate. To obtain cytosolic acetyl CoA, many cells transport citrate to the cytosol, where it is cleaved to acetyl CoA and oxaloacetate by citrate lyase. cycle to keep running, cells have to supply enough four-carbon intermediates from degradation of carbohydrate or certain amino acids to compensate for the rate of removal. Pathways or reactions that replenish the intermediates of the TCA cycle are referred to as anaplerotic ("filling up").

1. PYRUVATE CARBOXYLASE IS A MAJOR ANAPLEROTIC ENZYME

Pyruvate carboxylase is one of the major anaplerotic enzymes in the cell. It catalyzes the addition of CO_2 to pyruvate to form oxaloacetate (Fig. 20.18). Like most carboxylases, pyruvate carboxylase contains biotin, which forms a covalent intermediate with CO_2 in a reaction requiring ATP and Mg^{2+} (see Fig. 8.12, Chap. 8). The activated CO_2 is then transferred to pyruvate to form the carboxyl group of oxaloacetate.

Pyruvate carboxylase is found in many tissues, such as liver, brain, adipocytes, and fibroblasts, where its function is anaplerotic. Its concentration is high in liver and kidney cortex, where there is a continuous removal of oxaloacetate and malate from the TCA cycle to enter the gluconeogenic pathway.

Pyruvate carboxylase is activated by acetyl CoA and inhibited by high concentrations of many acyl CoA derivatives. As the concentration of oxaloacetate is depleted through the efflux of TCA cycle intermediates, the rate of the citrate synthase reaction decreases and acetyl CoA concentration rises. The acetyl CoA then activates pyruvate carboxylase to synthesize more oxaloacetate.

2. AMINO ACID DEGRADATION FORMS TCA CYCLE INTERMEDIATES

The pathways for oxidation of many amino acids convert their carbon skeletons into 5- and 4-carbon intermediates of the TCA cycle that can regenerate oxaloacetate (Fig 20.19). Alanine and serine carbons can enter through pyruvate carboxylase (see Fig.20.19, circle 1). In all tissues with mitochondria (except for, surprisingly, the liver), oxidation of the two branched chain amino acids isoleucine and valine to succinyl CoA forms a major anaplerotic route (see Fig.20.19, circle 3). In the liver, other compounds forming propionyl CoA (e.g., methionine, thymine and odd-chain length or branched fatty acids) also enter the TCA cycle as succinyl CoA. In most tissues, glutamine is taken up from the blood, converted to glutamate, and then oxidized to α -ketoglutarate, forming another major anaplerotic route (see Fig.20.19, circle 2). However, the TCA cycle cannot be resupplied with intermediates by even chain length fatty acid oxidation, or ketone body oxidation, which forms only acetyl CoA. In the TCA cycle, two carbons are lost from citrate before succinyl CoA is formed, and, therefore, there is no net conversion of acetyl carbon to oxaloacetate.

Pyruvate carboxylase deficiency is one of the genetic diseases grouped together under the clinical manifestations of Leigh's disease (subacute necrotizing encephalopathy). In the mild form, the patient presents early in life with delayed development and a mild-to-moderate lactic acidemia. Patients who survive are severely mentally retarded, and there is a loss of cerebral neurons. In the brain, pyruvate carboxylase is present in the astrocytes, which use TCA cycle intermediates to synthesize glutamine. This pathway is essential for neuronal survival. The major cause of the lactic acidemia is that cells dependent on pyruvate carboxylase for an anaplerotic supply of oxaloacetate cannot oxidize pyruvate in the TCA cycle (because of low oxaloacetate levels), and the liver cannot convert pyruvate to glucose (because the pyruvate carboxylase reaction is required for this pathway to occur), so the excess pyruvate is converted to lactate.



Fig. 20.18. Pyruvate carboxylase reaction. Pyruvate carboxylase adds a carboxyl group from bicarbonate (which is in equilibrium with CO_2) to pyruvate to form oxaloacetate. Biotin is used to activate and transfer the CO_2 . The energy to form the covalent biotin– CO_2 complex is provided by the high-energy phosphate bond of ATP, which is cleaved in the reaction. The enzyme is activated by acetyl CoA.

Biotin is a vitamin. A deficiency of biotin is very rare in humans because it is required in such small amounts and is synthesized by intestinal bacteria. However, an interesting case of biotin deficiency arose in a man eating a diet composed principally of peanuts and raw egg whites. Egg whites contain a biotin binding protein, avidin. Since he did not denature avidin by cooking the egg whites, it depleted his diet of biotin.



Fig. 20.19. Major anaplerotic pathways of the TCA cycle. 1 and 3 (blue arrows) are the two major anabolic pathways. (1) Pyruvate carboxylase (2) Glutamate is reversibly converted to α -ketoglutarate by transaminases (TA) and glutamate dehydrogenase (GDH) in many tissues. (3) The carbon skeletons of valine and isoleucine, a 3-carbon unit from odd chain fatty acid oxidation, and a number of other compounds enter the TCA cycle at the level of succinyl CoA. Other amino acids are also degraded to fumarate (4) and oxaloacetate (5), principally in the liver.

CLINICAL COMMENTS

Otto Shape. Otto Shape is experiencing the benefits of physical conditioning. A variety of functional adaptations in the heart, lungs, vascular system, and skeletal muscle occur in response to regular graded exercise. The pumping efficiency of the heart increases, allowing a greater cardiac output with fewer beats per minute and at a lower rate of oxygen utilization. The lungs extract a greater percentage of oxygen from the inspired air, allowing fewer respirations per unit of activity. The vasodilatory capacity of the arterial beds in skeletal muscle increases, promoting greater delivery of oxygen and fuels to exercising muscle. Concurrently, the venous drainage capacity in muscle is enhanced, ensuring that lactic acid will not accumulate in contracting tissues. These adaptive changes in physiological responses are accompanied by increases in the number, size, and activity of skeletal muscle mitochondria, along with the content of TCA cycle enzymes and components of the electron transport chain. These changes markedly enhance the oxidative capacity of exercising muscle.

Ann O'Rexia. Ann O'Rexia is experiencing fatigue for a number of reasons. She has iron deficiency anemia, which affects both iron-containing hemoglobin in her red blood cells, iron in aconitase and succinic dehydrogenase, as well as iron in the heme proteins of the electron

In skeletal muscle and other tissues, ATP is generated by anaerobic glycolysis when the rate of aerobic respiration is inadequate to meet the rate of ATP utilization. Under these circumstances, the rate of pyruvate production exceeds the cell's capacity to oxidize NADH in the electron transport chain, and hence, to oxidize pyruvate in the TCA cycle. The excess pyruvate is reduced to lactate. Because lactate is an acid, its accumulation affects the muscle and causes pain and swelling. transport chain. She may also be experiencing the consequences of multiple vitamin deficiencies, including thiamine, riboflavin, and niacin (the vitamin precursor of NAD⁺). It is less likely, but possible, that she also has subclinical deficiencies of pantothenate (the precursor of CoA) or biotin. Because of this, Ann's muscle must use glycolysis as their primary source of energy, which results in sore muscles.

Riboflavin deficiency generally occurs in conjunction with other watersoluble vitamin deficiencies. The classic deficiency symptoms are cheilosis (inflammation of the corners of the mouth), glossitis (magenta tongue), and seborrheic ("greasy") dermatitis. It is also characterized by sore throat, edema of the pharyngeal and oral mucus membranes, and normochromic, normocytic anemia associated with pure red cell cytoplasia of the bone marrow. However, it is not known whether the glossitis and dermatitis are actually due to multiple vitamin deficiencies.

Al Martini. Al Martini presents a second time with an alcohol-related high output form of heart failure sometimes referred to as "wet" beriberi, or as the "beriberi heart" (see Chapter 9). The term "wet" refers to the fluid retention which may eventually occur when left ventricular contractility is so compromised that cardiac output, although initially relatively "high," cannot meet the "demands" of the peripheral vascular beds, which have dilated in response to the thiamine deficiency.

The cardiomyopathy is directly related to a reduction in the normal biochemical function of the vitamin thiamine in heart muscle. Inhibition of the α -keto acid dehydrogenase complexes causes accumulation of α -keto acids in heart muscle (and in blood), resulting in a chemically-induced cardiomyopathy. Impairment of two other functions of thiamine may also contribute to the cardiomyopathy. Thiamine pyrophosphate serves as the coenzyme for transketolase in the pentose phosphate pathway, and pentose phosphates accumulate in thiamine deficiency. In addition, thiamine triphosphate (a different coenzyme form) may function in Na⁺ conductance channels.

Immediate treatment with large doses (50-100 mg) of intravenous thiamine may produce a measurable decrease in cardiac output and increase in peripheral vascular resistance as early as 30 minutes after the initial injection. Dietary supplementation of thiamine is not as effective because ethanol consumption interferes with thiamine absorption. Because ethanol also affects the absorption of most watersoluble vitamins, or their conversion to the coenzyme form, Al Martini was also given a bolus containing a multivitamin supplement.

BIOCHEMICAL COMMENTS



Compartmentation of Mitochondrial Enzymes. The mitochondrion forms a structural, functional, and regulatory compartment within the cell. The inner mitochondrial membrane is impermeable to anions and cations, and compounds can cross the membrane only on specific transport proteins. The enzymes of the TCA cycle, therefore, have more direct access to products of the previous reaction in the pathway than they would if these products were able to diffuse throughout the cell. Complex formation between enzymes also restricts access to pathway intermediates. Malate dehydrogenase and citrate synthase may form a loosely associated complex. The multienzyme pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complexes are examples of substrate channeling by tightly bound enzymes; only the transacylase enzyme has access to the thiamine-bound intermediate of the reaction, and only lipoamide dehydrogenase has access to reduced lipoic acid.

Riboflavin has a wide distribution in foods, and small amounts are present as coenzymes in most plant and animal tissues. Eggs, lean meats, milk, broccoli, and enriched breads and cereals are especially good sources. A portion of our niacin requirement can be met by synthesis from tryptophan. Meat (especially red meat), liver, legumes, milk, eggs, alfalfa, cereal grains, yeast, and fish are good sources of niacin and tryptophan.

Beri-beri, now known to be caused by thiamine deficiency, was attributed to lack of a nitrogenous component in food by Takaki, a Japanese surgeon, in 1884. In 1890, Eijkman, a Dutch physician working in Java, noted that the polyneuritis associated with beri-beri could be prevented by rice bran that had been removed during polishing. Thiamine is present in the bran portion of grains, and abundant in pork and legumes. In contrast to most vitamins, milk and milk products, seafood, fruits, and vegetables are NOT good sources of thiamine.



Thiamine

"Now polished rice isn't nice", Said the Dutchman Eijkman. Whole grains are a far better Source of thiamine. For beri-beri is very, very Hard on your nerves, you see. Polyneuritis and an enlarged heart May both accompany A very bad diet, a very sad diet A diet thiamine-free. And many who dine, only on wine Or consume brandy, whiskey or gin May never recover, if you don't discover They can't absorb thiamine. Wernicke-Korsakoff describe the signs And the confusion in the minds Of patients with this deficiency. So good doctors remember, try to recall Before you charge your fee, To give an injection, im or iv Of this vitamin B.

revised from an anonymous author



Fig. 20.20. Model for the import of nuclearencoded proteins into the mitochondrial matrix. The matrix preprotein with its positively charged N-terminal presequence is shown in blue. Abbreviations: OM, outer mitochondrial membrane; IMS, intramembrane space; IM, inner mitochondrial membrane; TOM, translocases of the outer mitochondrial membrane; TIM, translocases of the inner mitochondrial membrane; mthsp70, mitochondrial heat shock protein 70. Compartmentation plays an important role in regulation. The close association between the rate of the electron transport chain and the rate of the TCA cycle is maintained by their mutual access to the same pool of NADH and NAD⁺ in the mitochondrial matrix. NAD⁺, NADH, CoASH, and acyl CoA derivatives have no transport proteins and cannot cross the mitochondrial membrane. Thus, all of the dehydrogenases compete for the same NAD⁺ molecules, and are inhibited when NADH rises. Likewise, accumulation of acyl CoA derivatives (e.g., acetyl CoA) within the mitochondrial matrix affects other CoA-utilizing reactions, either by competing at the active site or limiting CoASH availability.

Import of Nuclear Encoded Proteins. All mitochondrial matrix proteins, such as the TCA cycle enzymes, are encoded by the nuclear genome. They are imported into the mitochondrial matrix as unfolded proteins that are pushed and pulled through channels in the outer and inner mitochondrial membranes (Fig. 20.20). Proteins destined for the mitochondrial matrix have a targeting N-terminal presequence of about 20 amino acids that includes several positively charged amino acid residues. They are synthesized on free ribosomes in the cytosol and maintain an unfolded conformation by binding to hsp70 chaperonins. This basic presequence binds to a receptor in a TOM complex (translocators of the outer membrane) (see Fig. 20.20, step 1). The TOM complexes consist of channel proteins, assembly proteins and receptor proteins with different specificities (e.g., TOM20 binds the matrix protein presequence). Negatively charged acidic residues on the receptors and in the channel pore assist in translocation of the matrix protein through the channel, presequence first.

The matrix preprotein is translocated across the inner membrane through a TIM complex (translocases of the inner membrane) (see Fig. 20.20, step 2). Insertion of the preprotein into the TIM channel is driven by the potential difference across the membrane, $\Delta \psi$. Mitochondrial hsp70 (mthsp70), which is bound to the matrix side of the TIM complex, binds the incoming preprotein and may "ratchet" it through the membrane. ATP is required for binding of mthsp70 to the TIM complex and again for the subsequent dissociation of the mthsp70 and the matrix preprotein. In the matrix, the preprotein may require another heat shock protein, hsp60, for proper folding. The final step in the import process is cleavage of the signal sequence by a matrix processing protease (see Fig. 20.20, step 3).

Proteins of the inner mitochondrial membrane are imported through a similar process, using TOM and TIM complexes containing different protein components.

Suggested References

Robinson, BH. Lactic acidemia: Disorders of pyruvate carboxylase and pyruvate dehydrogenase. In: Scriver CR, Beudet AL, Sly WS, Valle D, eds: The Metabolic and Molecular Bases of Inherited Disease. Vol. I. 8th Ed. New York: McGraw-Hill, 2001:4451–4480.

REVIEW QUESTIONS—CHAPTER 20

1. Which of the following coenzymes is unique to α -ketoacid dehydrogenase complexes?

- (A) NAD^+
- (B) FAD
- (C) GDP
- (D) H_2O
- (E) Lipoic acid

- 2. A patient diagnosed with thiamine deficiency exhibited fatigue and muscle cramps. The muscle cramps have been related to an accumulation of metabolic acids. Which of the following metabolic acids is most likely to accumulate in a thiamine deficiency?
 - (A) Isocitric acid
 - (B) Pyruvic acid
 - (C) Succinic acid
 - (D) Malic acid
 - (E) Oxaloacetic acid
- 3. Succinate dehydrogenase differs from all other enzymes in the TCA cycle in that it is the only enzyme that displays which of the following characteristics?
 - (A) It is embedded in the inner mitochondrial membrane.
 - (B) It is inhibited by NADH.
 - (C) It contains bound FAD.
 - (D) It contains Fe-S centers.
 - (E) It is regulated by a kinase.
- 4. During exercise, stimulation of the tricarboxylic acid cycle results principally from which of the following?
 - (A) Allosteric activation of isocitrate dehydrogenase by increased NADH
 - (B) Allosteric activation of fumarase by increased ADP
 - (C) A rapid decrease in the concentration of four carbon intermediates
 - (D) Product inhibition of citrate synthase
 - (E) Stimulation of the flux through a number of enzymes by a decreased NADH/NAD⁺ ratio
- 5. Coenzyme A is synthesized from which of the following vitamins?
 - (A) Niacin
 - (B) Riboflavin
 - (C) Vitamin A
 - (D) Pantothenate
 - (E) Vitamin C